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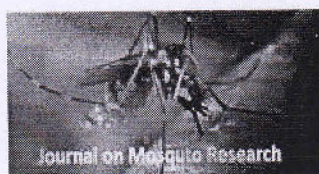
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Effect of alprazolam on the developmental stages of forensically important blow fly *Chrysomya megacephala* (Diptera: Calliphoridae)

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Abstract

In the present investigation impact of sedative drug alprazolam on growth rate and development cycle of *Chrysomya megacephala* (Diptera: Calliphoridae) was studied. The life cycle of *Chrysomya megacephala* includes egg, larva (three instars), pre-pupa, pupa and adult stages. Alterations in the life cycle of *Chrysomya megacephala* species was studied after exposure to alprazolam. The alprazolam treated food affect on growth rate of the larvae. The growth rate of *Chrysomya megacephala* was delayed in larvae fed with alprazolam containing diet. As the concentration of alprazolam increases in the food the larval and pupal development slows down. The flies appear first from control then from 0.4 ppm, while in higher concentrations of alprazolam 1.6 ppm, the pupation was delayed.

Keywords: Calliphoridae, *Chrysomya megacephala*, alprazolam, life cycle

1. Introduction

Forensic entomology is the scientific study of arthropods for investigation of crimes. In certain cases it is difficult to determine time of death beyond a certain postmortem period. In such cases length of time that insects have colonized on dead bodies is useful to determine time of death. Blow flies are the significant insects to determine time of death because the time they begin to colonize on dead bodies is many times approximately consistent with the time of death. The postmortem interval (PMI) has main importance for crime investigating agencies and judicial system throughout the world. Larvae of necrophagous fly are used for the determination of PMI, because these flies are the first insect to reach the dead body within few minutes to some hours after death [15].

The blow flies begin their life as eggs that are usually laid in large numbers on decaying material. The egg hatch into first-instar larvae, the maggots pass through three larval stages and ready to pupate. The mature maggots migrate in the soil to pupate, finally adult blow flies emerge to begin the life process again. Blowflies usually arrive at a corpse within few hours of death and lay their eggs [11].

Sedative drugs are the substance that used to treat anxiety, panic disorders, sleep disorders, tension but higher doses may result in poor judgment, staggering gait, slurred speech, unconsciousness and even death. Alprazolam is a benzodiazepine, it works by enhancing the activity of certain neurotransmitters in the brain, it is used to treat anxiety disorders panic attacks and depression. The misuse of such compounds has frequently been reported sadly and is related with increased mortality [8, 9]. Alprazolam is more toxic in overdose as compared to other benzodiazepines. Combined overdose with alcohol, tricyclic antidepressants, opioid medicine or other drugs can slow or stop your breathing and fatal side effects can occur. Benzodiazepines are generally seen in post-mortem samples because it is commonly used for suicide [10]. Some studies have showed that the developmental rate of insects can be affected by presence of drugs in decomposing tissues [12, 13, 14, 20].

Forensic entomology-toxicology is well known method of estimation of postmortem interval it includes the study of effects of drugs and toxins on developmental cycle of carrion-feeding insects. Analysis of living material such as larvae has number of practical advantages for drug detection than putrefied human remains. The presence of the drugs in the dead tissues of animal can affect on the growth rate and developmental cycle of the insects of forensic

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importance hence it is necessary to study the effect of alprazolam on growth rate and developmental stages of blow flies.

2. Materials and Methods

Chrysomya megacephala (Diptera: Calliphoridae) species were collected from decaying meat in the Aurangabad region. The flies does not need the flesh of specific animal, these flies occurs on the dead human body, also occurs on the flesh of any animal and hence for the study goat or other available flesh in the market was used. After one day of putrefaction, the liver and other meat was placed in open air for collection of flies. After some time the flies gathered on the putrid meat. The flies of calliphoridae family were collected by insect collecting net after identification of *Chrysomya megacephala* they were released in insect rearing cages.

2.1 Treatment of Alprazolam

The flesh was finely chopped in the mixer and was mixed with the alprazolam so as to make the concentration as 0.4 ppm, 0.8 ppm, 1.2 ppm and 1.6 ppm. Each of the alprazolam mixed flesh is placed in separate culture chambers, one sample of flesh was maintained as control. Fresh chopped meat was provided twice a day as food. Honey soaked in cotton was provided as the source of sugar and glucose. Wet cloth picce was also maintained on one side of the cage to maintain the humidity. The feed was changed on every day and the mortality was recorded.

The developing stages were collected on every day; maggots were narcotized in menthol water and were stored in vials containing AGA solution (alcohol, glycerol and acetic acid). Narcotization inactivates the maggots at relaxed condition and

hence after preservation there is no contraction of the maggots. The vial was labeled as the stage, date and time of collection. The different stages were photographed and weighed on the electronic balance. Measurements of these stages were made by the microscope whose least count is 0.001. The temperature and humidity were recorded at the same time. Measurements of five maggots were done at each time and their average with the standard deviation is calculated. Difference between the mean values of control and treated were analyzed by Student's t-test. Difference were considered statistically significant when, $p < 0.05$. The data obtained is tabulated in the table for different groups.

3. Results and Discussion

For studying development rate of *Chrysomya megacephala* the flies were collected from decaying flesh in Aurangabad region. The morphometric parameters namely length, width and weight for larvae and pupae is measured on flesh treated with alprazolam and compared to those fed on untreated flesh. The eggs of *Chrysomya megacephala* were collected on first day and then thirty eggs were placed separately on 0.0 ppm (control), 0.4 ppm, 0.8 ppm, 1.2 ppm and 1.6 ppm alprazolam containing chopped flesh. The observations were recorded each day with respect to the dose of concentrations (table 1). The temperature and humidity variations in the room conditions at the time of experiment are also mentioned (table 1). The larvae first emerged from control then from 0.4 ppm while at higher concentrations (1.6 ppm) pupation was delayed. The results showed that as the concentration of alprazolam increases in the food the larval development slows down and pupal development was delayed (table 1).

Table 1: Effect of Alprazolam on the Morphometric parameters of life cycle stages of *Chrysomya megacephala*.

PMI Days	Stages	Conc. of Diazepam	Length (mm)	Width (mm)	Weight (mg)	Temperature °C			Humidity %		
						Max	Min.	Recorded	Max	Min.	Recorded
1	I st Instar	Control	3.0±0.28	0.7±0.02	03±0.11	38.4	31.3	35.3	36	12	25
	I st Instar	0.4 ppm	3.0 ^{NS} ±0.32	0.7 ^{NS} ±0.041	03 ^{NS} ±0.12						
	I st Instar	0.8 ppm	2.9 ^{NS} ±0.26	0.7 ^{NS} ±0.045	03 ^{NS} ±0.13						
	I st Instar	1.2 ppm	2.9 ^{NS} ±0.27	0.6 ^{NS} ±0.034	02*±0.05						
	I st Instar	1.6 ppm	2.8*±0.25	0.5*±0.053	02*±0.08						
2	II nd Instar	Control	6.0±0.42	1.5±0.03	13±0.62	37.3	30	34	38	14	27
	II nd Instar	0.4 ppm	6.0 ^{NS} ±0.45	1.4 ^{NS} ±0.03	13 ^{NS} ±0.61						
	II nd Instar	0.8 ppm	5.9 ^{NS} ±0.40	1.4 ^{NS} ±0.032	12 ^{NS} ±0.57						
	II nd Instar	1.2 ppm	5.8*±0.38	1.3*±0.028	11*±0.53						
	II nd Instar	1.6 ppm	5.6**±0.35	1.2*±0.026	10*±0.43						
3	III rd Instar	Control	8.0±0.51	1.8±0.08	27±1.12	35.6	29	32	42	17	30
	III rd Instar	0.4 ppm	8.0 ^{NS} ±0.55	1.8 ^{NS} ±0.07	26 ^{NS} ±1.02						
	III rd Instar	0.8 ppm	7.9 ^{NS} ±0.48	1.7 ^{NS} ±0.071	25 ^{NS} ±1.01						
	III rd Instar	1.2 ppm	7.8*±0.45	1.6*±0.06	24*±1.17						
	III rd Instar	1.6 ppm	7.6**±0.41	1.5*±0.05	22*±0.98						
4	III rd Instar	Control	10.1±0.63	2.5±0.091	34±1.27	34.4	28.1	31.2	43	18	32
	III rd Instar	0.4 ppm	9.9 ^{NS} ±0.62	2.4 ^{NS} ±0.080	33 ^{NS} ±1.21						
	III rd Instar	0.8 ppm	9.8*±0.61	2.3*±0.075	33 ^{NS} ±1.29						
	III rd Instar	1.2 ppm	9.7*±0.57	2.1*±0.071	31*±1.18						
	III rd Instar	1.6 ppm	9.6**±0.54	2.0**±0.064	30*±1.13						
5	Pre-pupa	Control	9.6±0.86	2.8±0.045	40±1.19	38.1	31.2	33.6	38	14	27
	Pre-pupa	0.4 ppm	9.5 ^{NS} ±0.82	2.8 ^{NS} ±0.048	39 ^{NS} ±1.17						
	Pre-pupa	0.8 ppm	9.5 ^{NS} ±0.81	2.7 ^{NS} ±0.041	38 ^{NS} ±1.18						
	Pre-pupa	1.2 ppm	9.4*±0.82	2.6*±0.035	37*±1.16						
	Pre-pupa	1.6 ppm	9.2**±0.73	2.3**±0.038	35*±1.11						
6	Pre-pupa	Control	9.4±0.74	2.9±0.048	39±1.18	39.1	31	34.3	37	13	26
	Pre-pupa	0.4 ppm	9.2*±0.63	2.8 ^{NS} ±0.047	37 ^{NS} ±1.17						
	Pre-pupa	0.8 ppm	9.2*±0.69	2.8 ^{NS} ±0.043	37 ^{NS} ±1.19						
	Pre-pupa	1.2 ppm	9.1*±0.71	2.7*±0.045	36*±1.15						
	Pre-pupa	1.6 ppm	9.0**±0.78	2.6**±0.039	35*±1.18						

7	Pupa	Control	8.6±0.59	3.0±0.032	37±1.37	36.2	29	32.7	39	12	28
	Pupa	0.4 ppm	8.5 ^{NS} ±0.58	3.0 ^{NS} ±0.034	36 ^{NS} ±1.30						
	Pupa	0.8 ppm	8.4*±0.54	2.8*±0.029	36 ^{NS} ±1.28						
	Pre-pupa	1.2 ppm	8.5 ^{NS} ±0.58	2.8*±0.028	35 ^{NS} ±1.18						
	Pre-pupa	1.6 ppm	8.4*±0.57	2.7*±0.028	33*±1.15						
8	Pupa	Control	8.1±0.52	3.0±0.031	35±1.32	35.4	28	33.1	36	12	27
	Pupa	0.4 ppm	8.0 ^{NS} ±0.50	3.0 ^{NS} ±0.035	35 ^{NS} ±1.28						
	Pupa	0.8 ppm	8.0 ^{NS} ±0.51	2.9 ^{NS} ±0.03	34 ^{NS} ±1.24						
	Pupa	1.2 ppm	8.1 ^{NS} ±0.53	2.8*±0.029	33 ^{NS} ±1.18						
9	Adult	Control	8.2±0.54	4.1±0.049	50±1.121	38.3	31.2	35.1	35	10	25
	Adult	0.4 ppm	8.1 ^{NS} ±0.53	4.0 ^{NS} ±0.048	48 ^{NS} ±1.18						
	Pupa	0.8 ppm	8.0*±0.51	2.8**±0.046	34**±1.15						
	Pupa	1.2 ppm	7.9*±0.52	2.7**±0.041	32**±1.13						
10	Adult	0.8 ppm	7.9*±0.50	2.6**±0.037	32**±1.12	37.7	30.8	34.8	36	11	26
	Adult	1.2 ppm	8.0*±0.53	3.8*±0.041	45*±1.12						
	Pupa	1.6 ppm	7.8*±0.48	2.6**±0.039	31**±1.11						
11	Adult	1.6 ppm	8.0*±0.54	3.8*±0.040	44*±1.10	37.3	30.1	34.6	38	13	27

Where, * $p < 0.05$ (Significant t test), NS- Not significant

It is observed that duration of life cycle in treated food is longer as compared to control same results were reported by Pawar [21]. The results showed significant variations in development of larvae between the control and treated conditions the results corresponding to the results obtained from the study of effects of diamethoate on development rate of forensic importance Calliphoridae flies [18]. Same results also have been reported in the study of effect of endosulfan on development rate of *Chrysomya rufifacies* by Mali [19]. Forensic entomotoxicology is relatively new branch of forensic entomology which investigates the effect of drugs and toxic substances on growth of necrophagous insects [17]. Effect of cocaine on development of the sarcophagid fly, *Boettcherisca peregrine* was studied by Goff *et al.*, [22]. The eggs of blow flies have forensic importance and have been studied in various parts of the world [6, 3, 4, 20]. Watanabe *et al.*, [7] reported that forensic entomologist estimate the post-mortem interval (PMI) by identifying species found in association with a corpse. The postmortem Interval (PMI) is estimated by using developmental data of blow flies [5]. Studies showed that different drugs effect on the development rates of maggot resulting in inaccurate estimation of PMI [2, 1]. Pawar [20] observed time taken for larval development of *Lucilia cuprina* (Diptera: Calliphoridae) which were fed with diazepam containing food was considerably longer as compared to control. Mali [19] studied effect of endosulfan on maggot development of the *Lucilia cuprina*, in which he observed endosulfan treated tissue takes more time for maggot development as compared to control. The larvae of *Chrysomya megacephala* fed on diet containing different concentrations of ketamine their length and weight in treated group was lower than control group [16].

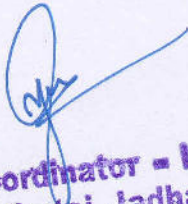
4. Conclusion

In forensic entomology-toxicology blow flies provide detail information about person's life before death when an individual has taken drugs. In the present study most commonly used sedative drug alprazolam were used to study their effect on developmental cycle of *Chrysomya megacephala*. The alprazolam affect on the developmental stages of *Chrysomya megacephala* and it has a direct impact on the estimation of PMI. Different stages of life cycle of blow flies and their morphometric parameters help in investigation of crimes.

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